GE Healthcare

IN Cell Analyzer

Advanced acquisition

Imaging slides, dishes, and chamber slides

1. When loading slide samples, coverslips must be affixed to the slide to be imaged with coverslip facing down. If coverslip is not affixed, load slide with coverslip facing up.
   Note: Coverslip must be facing down to use high numerical aperture (NA) objectives.

2. From the **Dashboard** in the IN Cell Analyzer acquisition software, select an appropriate slide or sample holder from the **Plate/Slide** drop-down.
   Note: If none of the available slide plate maps fit your sample, see application guide "Adding a plate map" (29258821).

3. If you are imaging slide(s), select the **Plate/Slide** card and click **Edit Plate**. In the **Cover Slip** section, select **Top** (coverslip facing up) or **Bottom** (coverslip down). Click **Apply** and **OK**.

4. Select the lowest magnification objective from the **Objective** drop-down.

5. Add the most stable or prominent channel in your sample using the **Add New Channel** [+] icon. Click **Auto Offset** to focus.

6. In **Plate View**, click the **Preview Scan** [icon] icon. Select an enlarged region around the sample area to define the scan area.

7. Click **Preview**.

8. After the scan completes, use **Plate View** to verify that the coverslip/sample area shown corresponds to the actual sample location (see example at right). Repeat Steps 6 to 7 for any additional samples.
   Note: If the plate/slide type selected does not accurately reflect your sample, see application guide "Adding a plate map" (29258821) to create a new plate map.

Correct   Incorrect

9. Set up protocol parameters as you would for a plate scanning experiment. Special considerations for slide imaging are detailed below:

   • In the **Fields** card, set up **Field Placement** as desired. The system will treat every coverslip as a well and image fields for each coverslip.

   • In the **Specimen Options** card, identify **Specimen location** and **Specimen thickness**.

Image processing—phase contrast and differential interference contrast (DIC)

1. Set up **Dashboard** parameters as you would for a plate scanning experiment.

2. Use the **Add New Channel** [+] icon to add **Brightfield** as the last channel.
   Note: To image in **Brightfield** you must remove any opaque covers (i.e., foil) from the sample.

3. In the **Channel Settings** card, select **Phase Contrast** or **DIC** from the **Image** drop-down for **Brightfield** channel.

4. In the **Image Processing** card, adjust **Phase Contrast** or **DIC** options as required:

   • **Phase contrast**

     » **High pass filter:** low values result in a smooth final image while high values accentuate fine details.

     » **Invert phase polarity:** objects in phase contrast images are generally dark with a bright halo. Checking this box inverts the image, resulting in light objects with a dark halo.

   • **DIC**

     » **Contrast angle (degrees):** changes the simulated angle of illumination, effectively shifting the direction of shadow relief in the image.

     » **Combiner prism (degrees):** A value of 180 degrees will result in the strongest DIC effect. Values close to 90 or - 90 degrees will look more like a regular transmitted light image.

5. Finalize any other protocol parameters and run as you normally would.

Time series

1. Set up **Dashboard** parameters as you would for a plate scanning experiment.

2. In the **Fields** card, select **Vertical** (raster) **Acquisition Pattern** for best repeatability.

3. In the **Time Series** card, check the **Acquire time** series box.

4. Enter values for **Starting Time Point**, **Time Interval**, and Number of **Time Points**. Click **Add**.
   Note: Starting Time Point is normally set to 0 unless it is necessary to allow time for sample temperature to equilibrate.

5. Select **Mode**. See table below for more information on time series modes.

6. If there is significant idle time between time points and sample heating is activated, check the **Incubate between time points** box to move the plate over the heater during idle time.

7. If autofocus is required, check the **Refocus at each Time Point** box.

8. Use checkboxes in the time series table to exclude time points or channels from the experiment. Right-click on the table for more selection/deselection options.

9. Finalize any other protocol parameters and run as you normally would.

Mode	Details	Use when
Single-well	Divides the wells into sub-groups to enable shorter time intervals	• Interval is too short to image more than one well • Imaging fast kinetic assays
Multi-well	Divides the wells into sub-groups to enable shorter time intervals	• Time interval is long enough to image more than one well at a time, but too short to image the whole plate
Multi-well*	Acquires all wells before moving to the next time point	• Interval is long enough to image all wells • Precise interval is not required (system will not notify if interval is too short to image all wells)
As fast as possible	Like single-well mode, but without delay between time points	• Fast frame rates are required for an extended period
Burst mode	Like single-well mode, but designed for short bursts	• Absolute maximum frame rate is needed • Precise timing is not required (time between images can be inconstant)

Merge image stacks

The **Merge Image Stacks** tool consolidates separate scans into a single multi-time point experiment. The tool is useful for experiments where time intervals are long or when pipetting or incubation between time points is required.

1. Select **Application/Merge Image Stacks**.
   Note: To merge experiments, all imaging protocols must be identical.

2. Click **Add** and navigate to experiment folder. Add two or more image stacks to be merged.
   Note: Stacks can be added in any order. Camera time stamps are used to assemble the merged stack sequentially.

3. Click **Next** and **Merge**. Merged files will be stored in a new folder. Original files are not deleted.